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Validating the use of sputum in the context of novel highly-sensitive molecular methodologies

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Increasingly, traditional culture-based diagnostic techniques are being overtaken by highly sensitive molecular methodologies. These new approaches are identifying an increasingly large number of species colonising the CF lung. It is therefore necessary to re-examine the susceptibility of sputa to contamination by organisms from the mouth and upper airways.

Automated T-RFLP analysis was used to compare the composition of bacterial communities in sputa from adult CF patients with those present in mouthwashes from the same individuals. Sputum and mouthwash samples from 20 adult CF patients were examined and data derived used to determine relative similarity.

Significant populations of bacteria, present in mouthwash samples, were not detected in the corresponding sputum sample. Further, many of the species detected in the sputum samples are not significantly represented in mouthwashes. The T-RFLP profiles generally grouped according to patient rather than sample type, strongly suggesting that the bacterial communities present at a location within the respiratory tract of individuals influence the community present at a separate location of their respiratory tract. However, the bacterial species most dominant in mouthwash samples are not typically those most prevalent within the lung.

This study shows that contamination of washed sputum samples by upper airway bacteria not originally present within the specimen is negligible. Further, these data reinforce our conclusion from previous studies that the wide range of species detected in the CF lung by molecular based approaches are true lung colonisers.

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Correlation of sinus, upper and lower airway cultures in CF children

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Aim: The aim of this study was to evaluate concordance of intraoperatively obtained sinus, oropharyngeal swab (OP) and bronchoalveolar lavage (BAL) cultures in CF children. **Methods**: CF children undergoing sinus surgery with concomitant BAL were included. An OP swab was obtained prior to anaesthesia, BAL prior to sinus surgery and sinus cultures during surgery. Concordance of pathogens was compared between these sites and in cases of the same organisms genetic identity was evaluated by pulse field gel electrophoresis (PFGE). **Results**: Forty-six surgery/BAL culture pairs in 31 patients were included. Twenty-six of these had matched OP culture. Mean age of patients at time of surgery was 9.5 ± 0.6 years and 15 patients were homozygote and 16 heterozygote for DF508. Bacterial sinus infection with one or more organisms was present in 96% of patients. Pathogens isolated from the sinuses were: *S. aureus* (50%), *P. aeruginosa* (Pa) (39%) and *H. influenzae* (22%). The same bacterial species were predominant in BAL and OP cultures, but 31% OP and 32.7% BAL were culture negative. Concordance of sinus and BAL was seen in 22% of *S. aureus* and 15% of Pa infections. Concordance of sinus and OP was slightly higher for *S. aureus* (27%) but same for Pa. All patients who had Pa only in sinus had history of Pa in the past OP or BAL cultures. To date, PFGE was done on 21 isolates obtained from 7 patients showing genetic identity in 86% of samples. **Conclusions**: In contrast to adult CF patients, BAL and OP cultures are poor predictors for organisms present in the sinuses of CF children. Antibiotic therapy of sinusitis in children with CF should include anti-staphylococcal coverage, but anti-pseudomonal therapy may be needed if the patient has a history of Pa infection in any culture.

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Correlation between microbiology profile from upper and lower respiratory tract of cystic fibrosis patients

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Aims: To determine whether there is relation between upper and lower airways microbiology in patients with cystic fibrosis (CF)

Methods: The design was a prospective transversal study involving 32 CF patients from the Cystic Fibrosis Group of University Hospital – UNICAMP – Brazil, between January 2003 and December 2004. The diagnosis was established on the basis of positive sweat test (> 60 mEq/L) and/or genotypic mutation. Maxillary sinus and tracheal secretion samples for bacterial cultures were obtained during endoscopic sinus surgery. Previously, oropharyngeal swab was recovered with an interval of 1 week from sinus surgery.

Results: The prevalence of the P.aeruginosa in oropharyngeal swab was 81,2% and in maxillary sinus and trachea, 31%. H.influenzae (56,2%) and S.aureus (75%) were collected from oropharynx and 18,7% and 68,7% respectively from maxillary sinus. 3 isolates of B.cepacia were collected in oropharyngeal swab, trachea and maxillary sinus. A. maltophilia and A.xylosidans were positive in 1 patient respectively and were positive in oropharynx swab, maxillary sinus and trachea.

Conclusions: oropharyngeal swab samples were of equal value as specimens from maxillary sinus and trachea, to detect bacterial colonisation.

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Relationship between iron deficiency and *Pseudomonas aeruginosa* vs. non-pseudomonal bacterial infections

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Aim: To determine whether there is a difference in iron status in CF patients with *Pseudomonas aeruginosa* infection (Psa) compared to other bacterial infections (non-Psa).

Method: A retrospective chart review was performed. 32 of the 70 adult patients had sputum microbiological cultures, lung function, serum iron and ferritin concentrations, TIBC, blood count, C-reactive protein (CRP) and erythocyte sedimentation rate measured at the same time. Iron status was compared among patients with mucoid (muc) Psa, non-mucoid (nmc) Psa, and non-Psa (S.aureus, Steno. maltophilia, Asp. fumigatus)

Results: 28 of the 32 patients had a Psa infection of which 61% had a transferrin saturation of <16% compared to 25% of those with non-Psa ($p<0.001$). Mean transferrin saturation was 12.4% (SD 4.5), 12.1% (4.2) and 21.0% (10.3) for those with muc Psa, nmc Psa and non-Psa, respectively ($p<0.05$). Serum iron levels were significantly lower in muc Psa (7.43µmol/L, SD 2.93) and nmc Psa (8.22µmol/L, SD 2.86) compared to non-Psa (15.5µmol/L, SD 9.33 $p<0.05$). Serum ferritin was significantly higher but normal in muc Psa (41.43µg/L, SD 20.30) vs. nmc Psa (18.20µg/L, SD 9.74) and non-Psa (19.75µg/L, SD 5.31) ($p<0.01$). TIBC was not significantly different among groups. Lung function was lower in muc Psa (35.2% predicted FEV_1, SD 9.0) vs. non-Psa (78.5%, SD 39.3%) ($p<0.05$). CRP was correlated to serum iron ($r_s=-0.62$, $p<0.01$), transferrin saturation ($r_s=-0.53$, $p<0.05$) and lung function ($r_s=-0.51$, $p<0.05$).

Conclusion: The results demonstrate that although all the bacteria require iron and have iron transport systems, Psa appears to have a greater negative impact on patients' iron status. The safety of iron supplementation in patients with Psa infection should be questioned.